PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Lasse LEINO et al.

Serial Number: 10/534,988 Group Art Unit: 1614

Filed: May 16, 2005 Examiner: Simmons, Chris E.

For: PHARMACEUTICAL COMPOSITION FOR INTRACELLULAR ACIDIFICATION WITH CIS-UROCANIC ACID

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jarmo LAIHIA, declare as follows:

1. I am one of the inventors of the invention described and claimed in U.S. Patent Application S.N. 10/534,988 ("the application") and am aware the claims of the application have been rejected as unpatentable over U.S. Patent No. 5,494,676 to Stab et al. in view of WO 02/07520 to Wei et al. and as unpatentable over Ben-Basset et al. in view of WO 02/07520.

2. I am the director of research and development for BioCis Pharma Oy, the assignee of the application. I, or those working under my supervision, performed experiments to measure

A. the irritation effect of pH of aqueous solutions in the skin of healthy human subjects and

B. the effect of Ben-Basset et al.'s compound AG 18 on the intracellular pH of neutrophils from the human peripheral blood.

A.1. A series of aqueous test solutions adjusted to 14 different pH values between pH 3.5 and pH 10 were prepared by mixing 0.05 M citric acid ($C_6H_8O_7$), 0.05 M disodiumhydrogen phosphate (Na_2HPO_4), and 0.05 M boric acid ($B(OH)_3$) in ultrapure water. Fifty microliters of each test solution was used to saturate a circular filter paper (11 mm in diameter, equalling 0.95 cm^2; Epitest, Tuusula, Finland) that were applied to the volar forearm skin of four apparently healthy individuals (two females and two males) under occlusion using Finn Chamber® aluminum chambers (Epitest). The chambers were attached with Scanpor® (Alpharma, Oslo, Norway) adhesive skin tape. After 24 h, the tape and the chambers were removed, and the skin was allowed to stabilize at room temperature for 20 min. For comparison, a positive irritation reaction was induced by applying 50 µl of 1 % sodium lauryl sulphate (SLS) in ultrapure water for 24 h (Gloor et al., Skin Res Technol 2004; 10: 144-8) under occlusion. A negative background control, corresponding to the irritation impact of the test procedure itself was 50 µl of

ultrapure water under occlusion.

A.2. The degree of redness (erythema), scaling, and fissures were evaluated according to visual scoring by Baranda et al. (Int J Dermatol 2002; 41: 494-9). Immediately after visual scoring, two consequent non-invasive measurements for local irritation/inflammation were performed in the treated spots and in an untreated control skin site. Skin redness was determined with UV-Optimize Matic 555 (Matic, Herlev, Denmark) which measures skin reflectance at the 660 nm wavelength. Transepidermal water loss (TEWL) was determined with the VapoMeter device (Delfin Technologies, Kuopio, Finland) as a measure of skin barrier integrity.

A.3. By visual scoring, no redness (erythema), scaling, or fissures (score zero in all cases) could be observed in any of the spots treated with the pH test solutions in the four individuals. As a positive control for comparison, speckling moderate (score 1+) to uniform moderate (2+) redness without scaling or fissures was observed in the SLS-treated skin sites. Graphs showing the average levels of measured erythema and TEWL after the pH solutions, SLS, and water treatments have been presented in Figure 1 below:

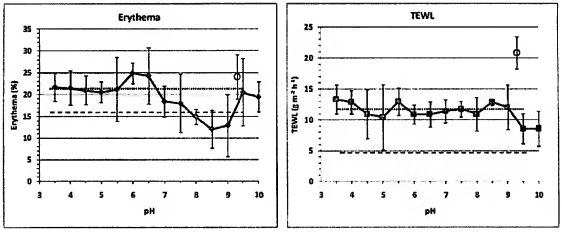

Figure 1. Skin erythema and TEWL measured by instrument methods (mean ± SD from four human individuals). The broken lines show the mean level measured in the untreated control skin and the dotted line the level in skin treated with purified water. The open circles denote the skin reaction caused by 1 % SLS solution.

A.4. According to visual scoring and TEWL, aqueous solutions over the pH range 3.5 to 10 do not induce irritation in the healthy human skin. In particular, no increase in erythema was detected by visual scoring at any pH.

Skin reflectance, as a measure of erythema, was the highest at pH 6.0 and 6.5. However, at higher pH values (pH 7-10) the erythema readings were below the water control. In conclusion, alkaline pH does not cause irritation in comparison to acidic pH, and thus there is no reason to adjust an alkaline pH to a lower pH to minimize skin irritation.

B.1. The effect of compound AG 18 on the intracellular pH was studied according to a methodology described in the application. Briefly, neutrophils were isolated from the peripheral blood of a healthy donor and loaded with the pH-sensitive fluorescent dye 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxy-fluorescein (BCECF, acetoxymethyl ester; Molecular Probes, Leiden, The Netherlands). Fifteen microliters of the loaded cell suspension was mixed with 0.3 ml of buffer solution containing 2.5, 10, 25, or 100 µmol/l AG 18 and adjusted at pH 7.4 or pH 6.5. The cells were kept at room temperature and analyzed by flow cytometry within 45 min.

B.2. The intensity of BCECF fluorescence at 525 nm was recorded for each sample, and the intracellular pH was determined by the fluorescence intensities of calibration cells prepared as explained in the application and buffered to pH 6.2, 6.5, 6.8, 7.2, 7.5, and 7.8. The experiment was performed similarly on three independent occasions.

B.3. The intracellular pH values calculated from the geometric mean fluorescence intensity in each cell sample have been shown in Figure 2 below:

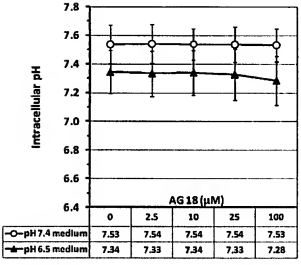

Figure 2. Intracellular pH in neutrophils after treatment with 2.5 to 100 µM AG 18. The data points in the graph are the mean ± SD from three independent experiments. The pH of the medium refers to the extracellular pH during the incubation with AG18.

B.4. The results show that AG 18 does not affect the intracellular pH of human peripheral blood neutrophils in physiological (pH 7.4) or mildly acidic (pH 6.5) extracellular medium in the concentration range 2.5 to 100 µM.

3. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Signed this the 30th day of March, 2009.

Signed:

Name: Jarmo Laihia